

Open Literature Review Summary

Chemical Name: Pymetrozine

CAS No: 23312-89-0

PC Code: 101103

Purpose of Review: Submission for Registration Review. MRID 45579701

Citation: Assessment of Side Effects of CGA 215944 50 WG (A-9364 A) on the Honey Bee (*Apis mellifera* L.) in the Field Following Application to *Phacelia tanacetifolia* during Bee-Flight in Spain. Study completed, Oct. 19, 2001. Study Code: 20011175/SI-BFEU

Date of Review: 7/14/17

Methods

Test Material: CGA 215944 50 WG (A-9364 A)

Guideline: Followed BBP Guideline VI. 23-1 and EPPO 170

Test species: Honey bee *Apis mellifera* (Hymenoptera, Apoidea) from single beekeeper in Spain.

Two trials (SOIN002B and SOIN003B) were carried out at two different test locations in Spain. One run was conducted in Northern Spain (Huesca; trial SOIN003B) and one further run in Middle Spain (Ayora; trial SOIN002B). CGA 215944 50 WG (A-9364 A) was tested at an application rate of 300 g product/ha in 500 L water/ha (nominally equivalent to 150 g a.i./ha in 500 L water/ha). The test item was applied with commercial equipment on fields of flowering *Phacelia tanacetifolia*. The effect of the application was examined on bee colonies used for honey production, which were placed near the test fields. A toxic standard (Dimethoate 40) was applied at a concentration of 0.9 L/ha in 200 L water/ha in one of the treatment locations (Note: due to a limitation, a toxic standard treated field was not available in the trial carried out in Northern Spain).

Colonies of comparable strength located at an untreated field with flowering *Phacelia* were used as control group. It is noted that the different fields of each trial were separated by at least 2 km to avoid bees foraging on other fields in that particular study and the field were not close to other nonflowering crops, which would be attractive to bees. On the day of application, the control fields were treated with 500 L water per ha. The effect of CGA 215944 50 WG (A-9364 A) was evaluated by comparing the bees of the test item treatments to the bee hives at the toxic standard field (trial SO I B002B) and the control fields and furthermore by comparing the pre- and post-application results of the following observations:

- Mortality in front of the bee hives and in the field
- Flight intensity in the field
- Behavior of the bees at the entrance of the hives
- Development of the bee brood
- Weight change of the colonies

Mortality and Flight Intensity

The mortality was measured using linen sheets in front of the hives and wooden traps with gauze on the bottom and top were attached to the entrance in order to record the number of dead bees. The flight intensity observations started three days before the application and in 1 m² marked locations regularly distributed on the test fields. At each assessment, the number of bees that are both foraging on flowering Phacelia and flying over the crop were counted for one minute per square meter.

Condition of Colony

- Strength of the colony (number of combs covered with bees)
- Presence of a healthy queen (presence of eggs, presence of queen cells)
- Estimate of the pollen storage area and area with nectar (in%)
- Estimate of the area containing eggs, larvae and capped cells (in%)
- Weight of the hives (DAA Oba, DAA 7 and 4 weeks) after the application.

At each assessment, both sides of one comb was assigned to be 100 % and the percentage area covered with the brood stages, pollen and nectar on the comb was estimated. This was done for all combs per hive. Afterwards the mean values were calculated for each hive and assessment date. Once the period of exposure ends (DAA 7), the hives were transferred to a location where pesticides are not used (forest area in the mountains) to ensure that further exposure to agrochemical was not possible.

Behavior Assessment and pollen collection

In addition to the assessments of mortality and flight intensity, the behavior of the bees returning to the entrance of the hives and during foraging in the crop was observed on the days before as well as after application at each time when the flight intensity assessments were carried out. The pollen retrieved by the bees was collected in front of one colony per treatment and subsequently identified. Therefore, a pollen trap was fixed in front of one hive (at least 3 hours) for three times during the test (DAA Oaa, DAA 2 and DAA 4).

Results:

The application of CGA 215944 50 WG resulted in a significant increase in honey bee mortality. On the day of application (DAA Oaa) an average of 51 dead bees/hive (counting bee trap and linen mortalities) was found in the test treatment compared to 2 dead bees/hive in the control and 493 for the toxic standard. Statistical analysis was conducted on the mortality reported via the bee trap and the results are reported in **Table 1**.

Table 1. Comparison of mortality in the dead bee traps (test item vs control) with the T-test (α 0.05, one sided higher) for each evaluation time, trial S01N002B

DAA	Statistical analyses of mortality		
	Test item	Toxic standard	Control
- 3	9.25 (+)	6.50 (-)	4.00
- 2	3.50 (-)	9.50 (-)	6.50
- 1	3.75 (-)	3.25 (-)	3.25
0 ba	0.75 (-)	0.25 (-)	0.50
After application			
0 aa	46.33 (+)	423.00 (+)	1.33
+ 1	16.75 (+)	171.00 (+)	1.25
+ 2	5.75 (+)	13.50 (+)	0.75
+ 3	6.25 (+)	10.50 (+)	0.50
+ 4	1.00 (-)	2.75 (+)	0.25
+ 5	0.75 (-)	2.00 (+)	0.25
+ 6	0.00 (-)	0.75 (-)	0.25
+ 7	0.25 (-)	1.25 (+)	0.00

(-): non-significant compared to the control

(+): significant compared to the control

DAA: days after application

ba = before application

aa = after application

For the second trial, on the day of application and on day 1 after treatment and increased bee mortality was observed in the test treatment with 63.7 and 82.8 dead bees per hive (based on bee trap and linen counts) compared to 8.7 and 10.3 dead bees/hive in the control. Statistical analysis was conducted on the mortality reported via the bee trap and the results are reported in Table 2.

Table 2. Comparison of mortality in the dead bee traps (test item vs control) with the T-test (0.05, one sided higher) for each evaluation time, trial S01N003B

DAA	Statistical analyses of mortality	
	Test item	Control
- 3	26.75 (-)	15.50
- 2	15.50 (-)	16.00
- 1	4.50 (-)	7.00
0 ba	1.25 (-)	1.75
After application		
0 aa	55.00 (-)	5.00
+ 1	73.50 (+)	7.25
+ 2	1.75 (-)	1.25
+ 3	2.75 (-)	3.00
+ 4	0.50 (-)	0.50
+ 5	1.00 (-)	1.50
+ 6	1.25 (-)	0.50
+ 7	0.25 (-)	0.25

(-): non-significant compared to the control

(+): significant compared to the control

DAA: days after application

ba = before application

aa = after application

Foraging activity/Flight intensity

Trial SOIN002B:

The daily average pre-application level of flight intensity was 14.5 bees/m² in the test item treatment, 18.7 bees/m² in the toxic standard, and 17.0 bees/m² in the control treatment. In the CGA 215944 50 WG (A-9364 A) treatment a slight decline of foraging activity was observed following the application which was restricted on that day. The number of foraging bees slightly decreased from 14.6 bees per m² flowering Phacelia immediately before application to an average of 9.2 bees/m² after treatment. In the control treatment the average flight intensity was 20.6 bees/m² directly before the application and was slightly reduced after the application on an average of 15.6 bees/m². In the toxic standard treatment, a clear decrease in flight intensity occurred on the day of treatment with 20.6 forager bees/m² before the application compared to an average of 7.7 bees/m² determined on the day of application after treatment. The average daily post-application level of flight intensity was 10.8 bees/m² in the test item treatment compared to 9.3 bees/m² in the toxic standard and 12.7 bees/m² in the control.

Trial S01N0038:

The average daily pre-application level of flight intensity was similar in both treatments with 8.4 bees/m²/minute in the test item treatment and 7.2 bees/m²/minute in the control treatment. In the test item treatment an average of 6.0 bees per m² flowering Phacelia was observed immediately before application compared to 3.6 bees/m² Phacelia in the control treatment. Directly after the application of the test item the

average flight intensity dropped to a lower level and remained there for the rest of the day (1.2 bees/m²). On the first assessment after the water treatment in the control the average flight intensity was similar as recorded before the application with 3.8 bees/m² noticed 55 minutes after treatment. From test day 1 to 3 after the application the average flight intensity in the test item treatment was decreased compared to the control treatment. On the following assessment days (DAA 4 -7), the average flight intensity was similar in both treatments.

Effects on honey bee brood development

There were no apparent treatment related effects observed for on honey bee brood development or colony weight.

Statistical Analysis

SAS Version 8 was used to analyze the mortality data for significant differences in comparison to the control. If differences were decided by the t-test, the homogeneity of the distribution was tested with Shapiro Wilk test.

Conclusion/Classification

Description of Use in Document (QUAL, QUAN, INV): Supplemental (QUAL)

Rationale for Use: This non-guideline study submission provides data that may be used for supplemental (qualitative) use in risk assessment.

Limitations of Study: At the last interval (day 31), there were decreases in colony weight across the control, toxic standard and treatment test colonies, thus, suggesting that sufficient forage was not available.

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